AD_____

Award Number: W81XWH-08-1-0034

TITLE: Humanized Androgen Receptor Mice: A Genetic Model for Differential Response to Prostate Cancer Therapy

PRINCIPAL INVESTIGATOR: Diane Robins

CONTRACTING ORGANIZATION: The University of Michigan
Ann Arbor, MI 48109

REPORT DATE: June 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-06-2011	Annual	15 APR 2010-14 APR 2011

4. TITLE AND SUBTITLE
Humanized Androgen Receptor Mice: A Genetic Model for Differential Response to Prostate Cancer Therapy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-1-0034

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Diane Robins

E-Mail: drobins@umich.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Michigan
Ann Arbor, MI 48109

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

In mice in which human androgen receptor (AR) replaces the endogenous murine gene, variation in the length of a polymorphic N-terminal polyglutamine tract affects initiation, progression and therapy response of prostate tumors. This provides a genetic paradigm in which to dissect AR functions that determine response to treatment. We are studying the role of the AR Q tract in ligand-independent AR activation in vitro and in a mouse model with prostate cancer ontogeny similar to human. In the mouse model, molecular correlates of differential response to castration will be determined using bioinformatic analysis of microdissected tumor samples. In the third year of this award, in in vitro studies, we showed that ARs with different Q tract lengths are differentially responsive to growth factor activation likely to be influential under castrate conditions. At least some of this differential activity appears to affected by phosphorylation at S-650. At this time, nearly all the experimental mice aged for tumorigenesis (containing a short or long Q-tract AR allele [12Q vs. 48Q], an ETV1 transgene and heterozygous loss of PTEN) have reached their time point and prostate samples have been collected for histology and biochemical analysis. Since tumorigenesis was much slower than anticipated, we performed some interim analysis on ETV1 transgenics wild type for PTEN to test AR efficacy. This will be an important control for the mice deficient in PTEN since both ETV1 and PTEN prove to directly influence AR action, which has relevance to human as well as mouse prostate cancer progression.

15. SUBJECT TERMS
androgen receptor, polyglutamine tract, mouse models, response to therapy

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	39	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Humanized Androgen Receptor Mice: A Genetic Model for Differential Response to Prostate Cancer Therapy

INTRODUCTION

Androgen works via the androgen receptor (AR) to drive prostate cancer (PCa) ontogeny and progression and therefore the androgen signaling pathway remains the primary therapeutic target. Such therapy is initially successful but tumors ultimately progress to hormone resistance. In these castration-recurrent tumors, AR levels remain high and AR signaling persists, reflecting that disease remains dependent on AR (1). To examine the role of AR in human disease, we created a mouse model by germ-line recombination in which human AR sequences replace those of the mouse (2, 3). Since a shorter N-terminal polyglutamine tract has been linked to PCa risk, alleles were created with 12, 21 or 48 glutamines (Q) to test association. The three "humanized" AR strains are grossly normal. Upon activation of a prostate-targeted oncogene however distinct allele-dependent differences in disease progression are evident. These allelic differences also impact progression following androgen ablation (4). Mice with the more active AR12Q respond well to castration, whereas those with the weaker AR48Q show no benefit from treatment. These strains provide a genetic paradigm in which to elucidate functions of AR that determine response to therapy. Identifying distinct pathways following treatment may reveal novel markers of therapy response and suggest differential strategies for optimal treatment. In this grant, we are studying the role of the Q tract in ligand-independent AR activation *in vitro* (Aim I) and, in a mouse model more similar to human cancer ontogeny, we will determine molecular correlates of differential response to castration (Aim II). In Aim III, mouse and human datasets will be compared to identify pathways signifying differential response to therapy.

BODY

The grant outlined 4 tasks in the Statement of Work. We have completed the first using a modified genetic strategy, have made significant progress on the second, and have accrued experimental animals necessary for the third with initial characterization of pathology. Relevant details, particularly those not previously reported, are described below.

Task 1. Establish mouse lines with both conditional PTEN and Q tract variant humanized AR (h/mAR) alleles.

We initially proposed conditional PTEN deletion (5) to initiate prostate tumorigenesis in C57BL/6 mice, for each h/mAR allele (12Q, 21Q, 48Q). Early in the project we modified the strategy to initiate cancer by global inactivation of one PTEN allele and cooperation of a prostate-targeted ETV1 transgene (6-8), on a FVB genetic background. This approach will provide more uniform oncogenesis than cre-mediated prostate-specific deletion of PTEN and the FVB background is less resistant to oncogenesis. The modified procedure and animal approval was obtained from U-M's UCUCA and from the DOD MRMC Animal Use Committee.

For each Q tract allele, 3 groups (**n>8**) will be compared – **pretreatment** (12 weeks of age), **untreated** (intact at 24-50 weeks) and **treated** (castrated at 12 weeks, aged to 24-50 weeks). An equivalent number of littermate controls without PTEN loss or lacking the ETV1 transgene will be examined. This model has proved to be much less aggressive than anticipated and thus the

mice are aged to 50 wks or until other tumors necessitate sacrifice. Upon reaching the time point (Task 3), prostates are microdissected and processed for histological and molecular analysis.

Task 2. Determine the role of the Q tract in ligand-independent androgen receptor (AR) activation in vitro.

The AR Q tract influence noted in mouse PCa (4) suggests differences in AR strength may contribute to differences in response to androgen ablation. Greater transcriptional activity of short Q tract ARs is due to greater interaction between AR's N and C terminus (N/C interaction), which leads to greater response at lower ligand levels and greater coactivator recruitment (9). AR can also be activated in a ligand-independent manner by growth factors, such as IGF, EGF, cytokines, PKA and tyrosine kinase receptors such as Her2/neu (10, 11). We tested whether growth factor signaling pathways are sensitive to Q tract length as a mechanism underlying Q tract effects in castration-resistant prostate cancer, and whether such sensitivity showed cell-type and promoter-specific preferences. Transient transfection of 12Q, 21Q and 48Q AR cDNAs in the presence of low or no hormone was used to explore differential activation, and ligand-independent activation was elicited by co-expression of a constitutively active growth factor, in low or no hormone. As reported last year, we confirmed our hypothesis that shorter Q tract ARs are hypersensitive to low ligand and show greater activation by growth factors. We showed distinct effects in RWPE-1 immortalized prostate cells (12) compared to tumorigenic PC-3 cells, and distinct effects on simple response elements versus complex promoters (13, 14). It is intriguing that AR activation that varied with Q tract length was significant in cancer but not normal cell lines, and more pronounced for AR binding sites alone than for the complex PSA promoter, likely due to compensatory effects of other DNA binding factors. That the cancer cell background accentuates the Q tract effect could be due to differences in other transcription factors or coactivator levels.

Last year we reported that ligand-independent AR activation by growth factor, modeled by constitutively active Raf-1 kinase (15), was sensitive to Q tract length. The effect was modest but inversely correlated with Q tract length as hypothesized. We tested other signaling pathways and found that neither Rac1 nor RhoA enhanced AR activation at castrate levels of androgen, and at higher hormone levels did not appear to be sensitive to Q tract length. It is possible that effects would be noted at earlier time points or under differing experimental conditions.

Since growth factor signaling often elicits post-translational modifications that influence protein activity and signaling through numerous pathways induces AR phosphorylation (16), we examined the effect of activated Raf-1 kinase on phosphorylation of Q tract variant ARs. We reported last year that phosphorylation of S81, adjacent to the Q tract, showed little effect of Q tract length but that S650 in the hinge domain was sensitive to ca-Raf1 phosphorylation in inverse correlation with Q tract length. This is intriguing since S650 is involved in DNA binding and coregulator contact (17, 18). To probe the outcome of this differential phosphorylation, we mutated the S650 site to mimic either constitutive phosphorylation (by a serine to glutamic acid [S → E] "phosphomimetic" amino acid substitution) or an inability to be phosphorylated (by an S → A change to a "phospho-dead" site). These mutant ARs were transfected as before into PC-3 cells and reporter activation assessed; data for the consensus HRE reporter is shown in Fig. 1. The phosphomimetic substitution (S650E) showed a pronounced influence on AR activity that was inversely correlated with Q tract length. This was pronounced at intact levels of hormone

but detectable also at castrate levels (0.01 nM R1881), particularly for the hypersensitive AR12Q. The non-phosphorylatable S650A mutants showed little difference from wild type (not shown), but interestingly AR48Q activity was greatly decreased by this mutation.

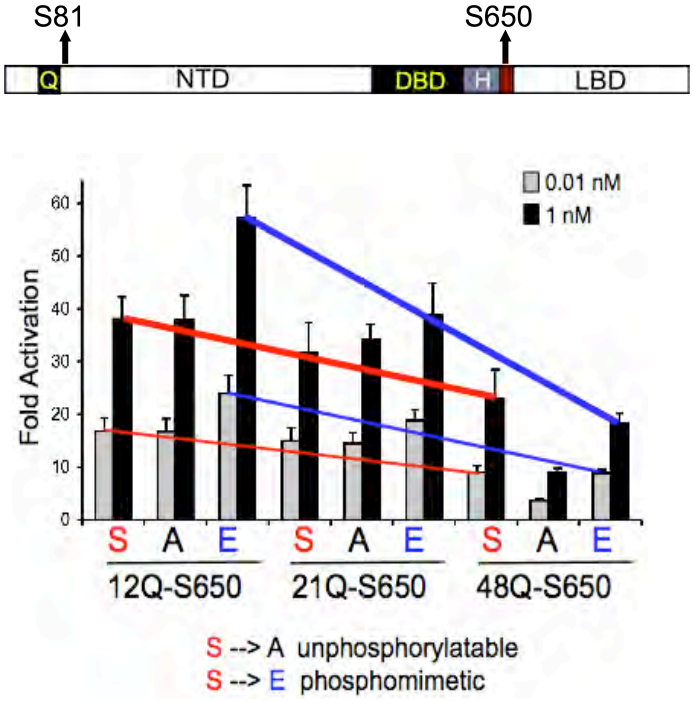

Fig. 1 – Activity of AR-S650 mutants depends on Q tract length. Variant Q tracts were introduced into AR21Q phosphorylation mutants in which S650 had been altered to A (unphosphorylatable) or E (phospho-mimetic). ARs were transfected into PC-3 cells and activation of 3xHRE3-luciferase was assayed after 24 hrs with 0.01 or 1 nm R1881. Relative luciferase activity was normalized to renilla and plotted as fold acvtivation over vehicle. The results of four independent transfections are shown. Red lines indicate activities for ARs that are wild type at S650; blue lines compare ARs with 650E mimicking phosphorylation. The modest difference in AR activity inversely dependent on Q tract length is enhanced by S650 phosphorylation.

This differential activity of Q tract variants dependent on phosphorylation at S650 is intriguing because phosphorylation at this site influences AR subcellular localization (18, 19). Receptor export is important for recycling and reactivation (20). Moreover, a coactivator sensitive to AR's Q tract length is RAN GTPase, which is involved in nuclear-cytoplasmic shuttling of numerous targets (21, 22). More efficient nuclear export of receptors may be linked to their greater hormonal response. The combination of phosphorylated S650 leading to rapid export and greater association with RAN allowing efficient nuclear reentry may account for greater activation in transfection by AR12Q.

Task 3. Determine the molecular correlates of androgen ablation response as affected by AR strength in prostate-specific PTEN-inactivated mice.

After some delays in breeding and use of longer time points due to slow progression of the model, almost all of the prostate samples have now been harvested. We anticipate successful completion of this project within the no-cost extension. Our new mouse model relies for tumorigenesis on PTEN heterozygosity with a "second hit" provided by a prostate-targeted ETV1 transgene (6, 23-25). Examination of a few pilot animals (reported last year) indicated we would need to extend the age of assessment beyond the 6 months initially planned. Because PTEN heterozygosity increases overall risk of cancer, we generated sufficient excess mice to account for some losses. The mice were examined every other day for palpable tumors so that diseased animals could be euthanized and prostate samples obtained. A small set of pilot animals examined at 10 months displayed significant prostate abnormality as well as differences between Q tract lengths (Fig. 2). From initial histological analysis, the ETV1 transgene appears

to increase the extent of hyperplasia and abnormality for all AR alleles. Following androgen ablation, hyperplasia appears to be less with the 12Q allele than the 48Q allele, as predicted by our earlier results in the TRAMP model. Currently these samples are with the pathologist for detailed histological analysis. These samples will be informative for the relationship between androgen axis strength and efficacy of androgen ablation. Since the model is less aggressive than anticipated, results may be relevant to prevention as well as to treatment response.

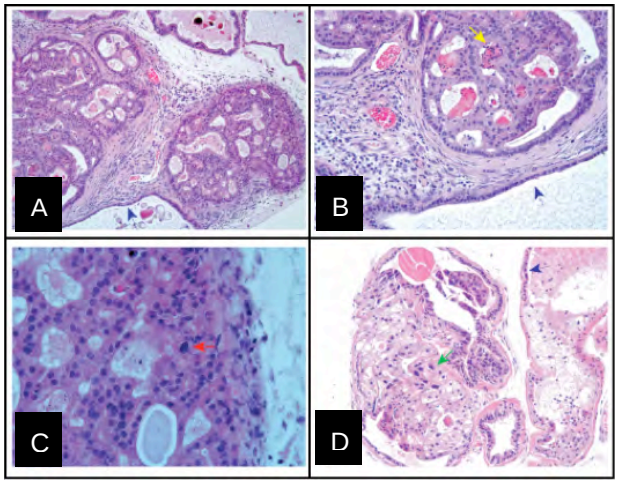

Fig. 2 – Pathology in Pten$^{-/+}$;Etv1^{tg} humanized AR mice. At sacrifice, prostates were microdissected into separate lobes, one of each pair of which was frozen for RNA analysis and one fixed, embedded, sectioned and stained by H&E. By 1 year, AR12Q mice show significant PIN and some cancer. The ETV1 transgene increases PIN and atypia, particularly in the dorsolateral (DLP) rather than ventral (VP) lobe. **A, B,** and **C** are H&E stained sections of an AR12Q Pten$^{-/+}$ VP; **D** is from an AR12Q Pten$^{-/+}$;Etv1^{tg} DLP. Arrows: blue – benign; yellow – microscopic necrosis; red – mitotic figure; green – cytologic atypia.

To examine interaction of AR and ETV1 without PTEN loss, i.e. events early in oncogenesis, and to optimize RNA methodology for subsequent samples, we analyzed a set of prostates from mice at 24 weeks of age that were either intact or had been castrated at 12 weeks. At 24 weeks, there was no evident histological difference dependent on the presence of the ETV1 transgene, or between AR12Q vs. AR48Q genotypes, although the effect of castration was obvious as expected. Interestingly, at the level of gene expression, profound differences were seen due to the presence or absence of the ETV1 transgene. A global view of gene expression differences is shown in the heat maps generated from microarray analysis (Fig. 3).

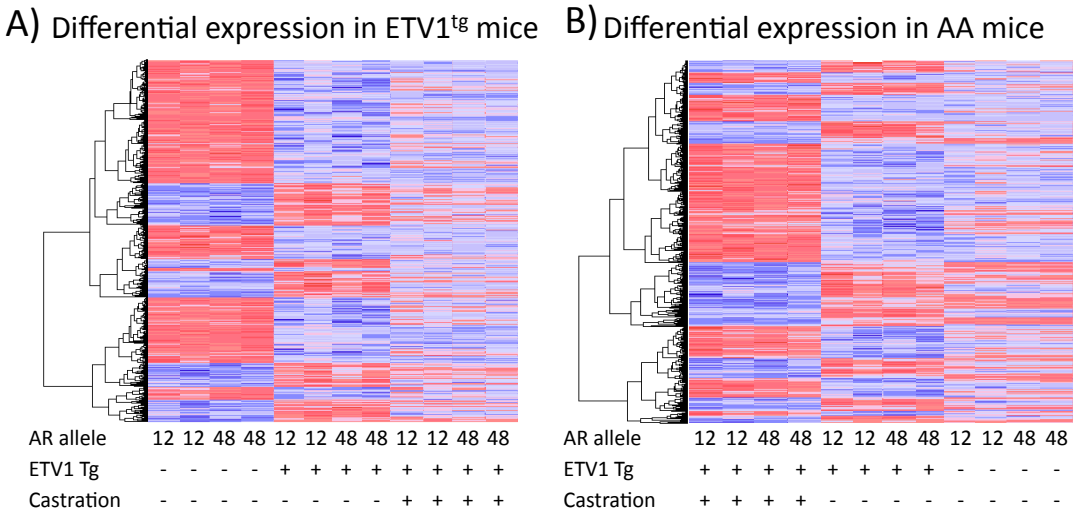

Fig. 3 – Effects of ETV1 Overexpression and Castration on Mouse Prostate Gene Expression. RNA from dorsolateral prostate lobes was analyzed on Affymetrix Mouse 430 2.0 gene expression microarrays at the U. of M. Microarray Core. Mice had either AR12Q or AR48Q alleles, and an ETV1 transgene driven by the ARR$_2$-probasin promoter as indicated. Mice were intact or castrated at 12 wks; prostates were harvested at 24 wks. RNA from 3-6 mice per group was divided into 2 pools, and each pool was analyzed on a separate microarray.

Differential gene expression between wild type mice and those with the ETV1 transgene was calculated from the microarray data, and individual genes with $p<0.001$ (1517 probesets) appear as horizontal lines on the heatmap (Fig. 3A), with genes with similar expression patterns grouped together. The relative expression for each sample is indicated by color, where red signifies high expression and blue low. To the right (Fig. 3B), intact and castrated mice are compared and graphed similarly; 1137 probesets reached significance ($p<0.001$). AR genotype had little global effect on gene expression, which agrees with our earlier data on normal as opposed to malignant prostates of the humanized mice (26). The ETV1 transgene had substantial impact, as did castration, each of which affected a comparable number of genes. Interestingly, when the top 5% of genes differentially affected by ETV1 (panel A) or castration (panel B) were analyzed with EASE software to test for Gene Ontology (GO) term enrichment, the top GO terms associated with ETV1 overexpression were primarily for metabolic processes, whereas the top GO terms for genes affected by androgen ablation were primarily associated with blood vessel development. This reflects cell growth and proliferation driven by ETV1, in contrast to hypotrophy and involution driven by castration.

To validate the microarray results and explore the interaction of AR and ETV1 regulatory pathways, expression of specific genes was examined by qRT-PCR (Fig. 4). We examined

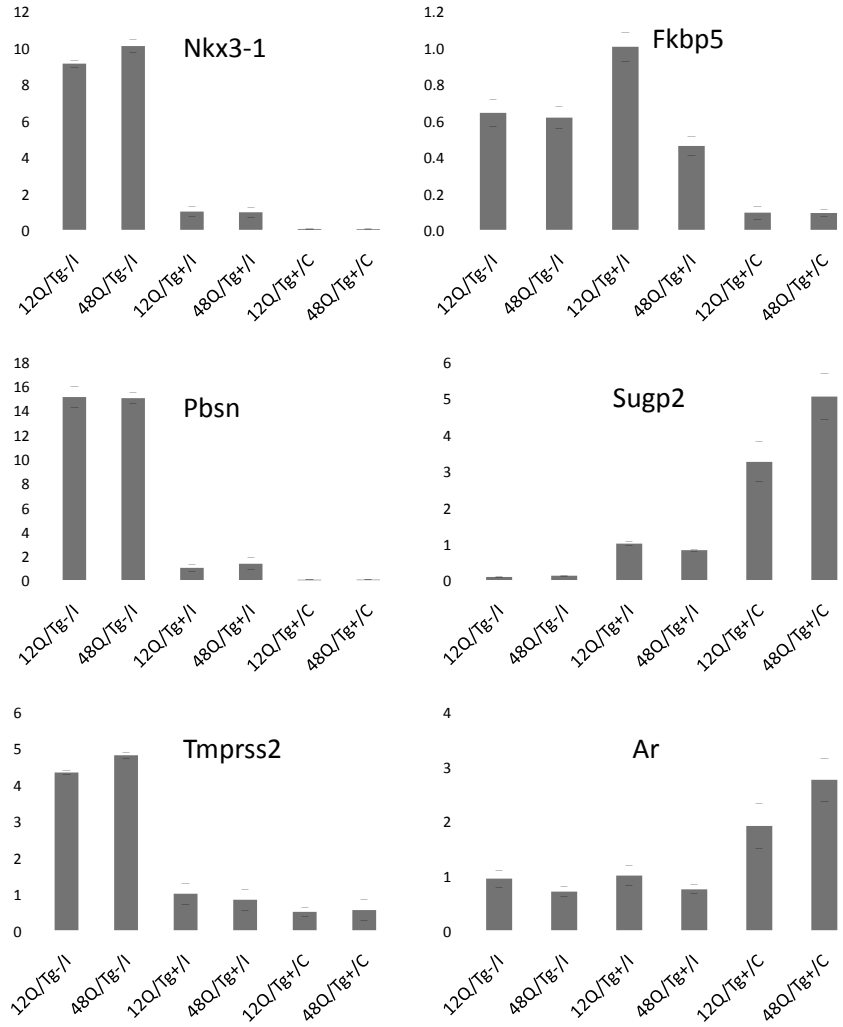

Fig. 4 – ETV1 transgenic mice show altered expression of AR target genes. Expression of key AR target genes was assayed by qRT-PCR of RNA from dorsolateral prostate lobes. Genotypes and treatment groups are labeled by Ar allele (12Q/48Q), presence of the ETV1 transgene (Tg+/Tg-) and intact vs. castrated (I/C). 3-6 mice per group were analyzed in triplicate reactions on an Applied Biosystems 7500 Real-Time PCR System using Power SYBR Green Master Mix. Relative expression was calculated using the ΔΔCt method with β-Actin as the reference gene. Error bars represent the standard error of the mean.

genes that are upregulated by AR (shown on the left in Fig. 4), including *Nkx3.1*, a homeobox gene critical in prostate development and oncogenesis, probasin (*Pbsn*) a marker of prostate differentiation analogous to human PSA, and *Tmprss2*, whose promoter is often fused upstream of ETS family genes in PCa, thus placing cell cycle regulation under androgen control. For these genes ETV1 overexpression appears to antagonize AR upregulation. For AR-repressed genes such as clusterin (*Sugp2*), the opposite pattern appears – repression by AR is less in the presence of ETV1. Differences dependent on AR allele (12Q vs. 48Q) are not significant for these targets. However, the androgen-responsive *Fkbp5* shows a rather distinct pattern – ETV1 cooperates with AR in expression of this gene for the 12Q but not the 48Q allele. *Fkbp5* has recently been shown to play a key role in PTEN-deficient tumors (24) and elucidating its interaction with the AR signaling pathway here in the ETV1 transgenic mice and subsequently in our PTEN cancer model should prove highly informative. Finally, the expression of AR itself is intriguing in these prostates – the 12Q allele shows modestly higher expression in intact mice and the 48Q in castrated mice. This could be due to either transcriptional effects or post-transcriptional control, such as differences in mRNA stability. Ultimately comparison to the dataset we will generate from the compound $Pten^{-/+}$; $ETV1^{tg}$ humanized AR mice should provide substantial information for delineating interaction of AR, PTEN and ETV1 pathways in PCa.

KEY RESEARCH ACCOMPLISHMENTS

- The Q tract variant h/mAR mice, heterozygous for PTEN and carrying the prostate-targeted ARR2PBi-ETV1 transgene have been derived and aged, with or without androgen ablation from 12 weeks, and most prostates have been harvested.
- Some of the Q tract effect on ligand-independent AR signaling may be due to phosphorylation of AR at Ser650, which correlates inversely with length of the Q tract and enhances transcription at low ligand to a greater extent for short Q tract ARs.
- Preliminary analysis of aged mice suggests this model will be informative albeit milder than expected – the ETV1 transgene demonstrably affects hyperplasia and leads to carcinoma when combined with PTEN deficiency.
- Microarray analysis of Q tract variant ETV1 transgenic mice at 24 weeks of age supports hypotheses of ETV1 and AR pathway interaction, and strengthens avenues for pursuit with the PTEN deficient mice.

REPORTABLE OUTCOMES

The following manuscripts benefitted from this project (the 1st ms. is appended):

1) Robins DM: Androgen receptor gene polymorphisms and alterations in prostate cancer: of humanized mice and men. Molecular and Cellular Endocrinology, invited review for special issue on androgen signaling, *in press*.

2) Simanainen U, Brogley M, Gao YR, Jimenez M, Harwood DT, Handelsman DJ, Robins DM: Length of the human androgen receptor glutamine tract determines androgen sensitivity in vivo. Molecular and Cellular Endocrinology, *in press*.

I presented Plenary addresses at two conferences that described previous work from the lab and mentioned new studies supported by this grant:

09/22/2010 – International Conference on Hormonal Steroids and Hormones & Cancer, Edinburgh, Scotland – Genetic Variation of the Androgen Receptor in Prostate Cancer

06/06/2011 – Endocrine Society Symposium, Boston, MA - Genetic Variation of the Androgen Receptor: from Gene Regulation to Prostate Cancer

A poster including data reported here was presented at the IMPaCT Meeting, 03/09/2011, Orlando, FL – "Differential Response to Androgen Depletion by Androgen Receptor Glutamine Tract Length Variation".

Based on work supported here, we obtained a small pilot award from the University of Michigan Center for Genomics in Health and Medicine to perform gene expression profiling of the Q tract mice with ETV1 transgenes (without PTEN loss), to compare microarray to RNA-seq analysis. Some of this work is discussed above and will enhance our future analysis of the PTEN mice.

Mouse strains and bioinformatic databases will be made publicly available once reported.

CONCLUSION

In this DOD IDEA award, we have constructed mouse strains in which to address the role of the AR Q tract in differential response to androgen ablation. In a more general sense, this model modulates the androgen axis, allowing us to elucidate a role of androgen sensitivity in cancer progression and treatment response. The homogeneous genetic background and genetic oncogenic paradigm similar to human prostate cancer (heterozygosity for PTEN and overexpression of ETV1) should amplify the subtle effects of AR variation. Prostates of experimental mice have been collected. Initial pathological analysis indicates that while tumorigenesis is slower than expected, AR Q tract differences are evident in intact as well as castrated mice. This suggests that bioinformatic analysis of gene expression will be informative in distinguishing markers of good versus poor response to castration therapy.

To examine molecular mechanisms underlying differential Q tract effects, we have performed cell-based assays. ARs with different Q tract lengths are sensitive to promoter and cell type differences, and are differentially sensitive to growth factor activation that may drive AR following androgen ablation. Subtle differences conferred by Q tract length may prove to be one of many factors that sum to significant affects in response to therapy.

REFERENCES

1. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer. 2001;1(1):34-45.
2. Albertelli MA, Scheller A, Brogley M, Robins DM. Replacing the mouse androgen receptor with human alleles demonstrates glutamine tract length-dependent effects on physiology and tumorigenesis in mice. Mol Endocrinol. 2006;20(6):1248-60.
3. Robins DM. Androgen receptor gene polymorphisms and alterations in prostate cancer: of humanized mice and men. Molecular and Cellular Endocrinology. 2011.
4. Albertelli MA, O'Mahony OA, Brogley M, Tosoian J, Steinkamp M, Daignault S, et al. Glutamine tract length of human androgen receptors affects hormone-dependent and -independent prostate cancer in mice. Hum Mol Genet. 2008;17(1):98-110.
5. Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J, et al. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell. 2003;4(3):209-21.
6. Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, et al. Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. Nat Genet. 2009;41(5):619-24.
7. King JC, Xu J, Wongvipat J, Hieronymus H, Carver BS, Leung DH, et al. Cooperativity of TMPRSS2-ERG with PI3-kinase pathway activation in prostate oncogenesis. Nat Genet. 2009;41(5):524-6.
8. Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature. 2007;448(7153):595-9.
9. Wang Q, Udayakumar TS, Vasaitis TS, Brodie AM, Fondell JD. Mechanistic relationship between androgen receptor polyglutamine tract truncation and androgen-dependent transcriptional hyperactivity in prostate cancer cells. J Biol Chem. 2004;279(17):17319-28.
10. Scher HI, Buchanan G, Gerald W, Butler LM, Tilley WD. Targeting the androgen receptor: improving outcomes for castration-resistant prostate cancer. Endocr Relat Cancer. 2004;11(3):459-76.
11. Wang G, Sadar MD. Amino-terminus domain of the androgen receptor as a molecular target to prevent the hormonal progression of prostate cancer. J Cell Biochem. 2006;98(1):36-53.
12. Bello D, Webber MM, Kleinman HK, Wartinger DD, Rhim JS. Androgen responsive adult human prostatic epithelial cell lines immortalized by human papillomavirus 18. Carcinogenesis. 1997;18(6):1215-23.
13. Perez-Stable CM, Pozas A, Roos BA. A role for GATA transcription factors in the androgen regulation of the prostate-specific antigen gene enhancer. Mol Cell Endocrinol. 2000;167(1-2):43-53.
14. Robins DM. Multiple mechanisms of male-specific gene expression: lessons from the mouse sex-limited protein (Slp) gene. Prog Nucleic Acid Res Mol Biol. 2004;78:1-36.
15. Mukherjee R, Bartlett JM, Krishna NS, Underwood MA, Edwards J. Raf-1 expression may influence progression to androgen insensitive prostate cancer. Prostate. 2005;64(1):101-7.
16. Gioeli D. Signal transduction in prostate cancer progression. Clin Sci (Lond). 2005;108(4):293-308.
17. Gioeli D, Black BE, Gordon V, Spencer A, Kesler CT, Eblen ST, et al. Stress kinase signaling regulates androgen receptor phosphorylation, transcription, and localization. Mol Endocrinol. 2006;20(3):503-15.

18. Gioeli D, Ficarro SB, Kwiek JJ, Aaronson D, Hancock M, Catling AD, et al. Androgen Receptor Phosphorylation. Regulation and identification of the phosphorylation sites. J Biol Chem. 2002;277(32):29304-14.
19. Ponguta LA, Gregory CW, French FS, Wilson EM. Site-specific Androgen Receptor Serine Phosphorylation Linked to Epidermal Growth Factor-dependent Growth of Castration-recurrent Prostate Cancer. J Biol Chem. 2008;283(30):20989-1001.
20. Kesler CT, Gioeli D, Conaway MR, Weber MJ, Paschal BM. Subcellular localization modulates activation function 1 domain phosphorylation in the androgen receptor. Mol Endocrinol. 2007;21(9):2071-84.
21. Hsiao PW, Lin DL, Nakao R, Chang C. The linkage of Kennedy's neuron disease to ARA24, the first identified androgen receptor polyglutamine region-associated coactivator. J Biol Chem. 1999;274(29):20229-34.
22. Hughes M, Zhang C, Avis JM, Hutchison CJ, Clarke PR. The role of the ran GTPase in nuclear assembly and DNA replication: characterisation of the effects of Ran mutants. J Cell Sci. 1998;111 (Pt 20):3017-26.
23. Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S, et al. Reciprocal Feedback Regulation of PI3K and Androgen Receptor Signaling in PTEN-Deficient Prostate Cancer. Cancer Cell. 2011;19(5):575-86.
24. Mulholland DJ, Tran LM, Li Y, Cai H, Morim A, Wang S, et al. Cell Autonomous Role of PTEN in Regulating Castration-Resistant Prostate Cancer Growth. Cancer Cell. 2011.
25. Shin S, Kim TD, Jin F, van Deursen JM, Dehm SM, Tindall DJ, et al. Induction of prostatic intraepithelial neoplasia and modulation of androgen receptor by ETS variant 1/ETS-related protein 81. Cancer Res. 2009;69(20):8102-10.
26. Albertelli M. Genetic Variation in the Androgen Receptor Impacts Prostate Cancer Initiation and Progression in the Humanized AR Mouse. Ann Arbor: University of Michigan Medical School; 2007.

Androgen Receptor Gene Polymorphisms and Alterations in Prostate Cancer:

Of Humanized Mice and Men

Diane M. Robins
Department of Human Genetics
University of Michigan Medical School
1241 E. Catherine St., 4909 Buhl
Ann Arbor, MI 48109-5618
USA

Phone: 1-734-764-4563
Fax: 1-734-763-3784
drobins@umich.edu

ABSTRACT

Germline polymorphisms and somatic mutations of the androgen receptor (AR) have been intensely investigated in prostate cancer but even with genomic approaches their impact remains controversial. To assess the functional significance of AR genetic variation, we converted the mouse gene to the human sequence by germline recombination and engineered alleles to query the role of a polymorphic glutamine (Q) tract implicated in cancer risk. In a prostate cancer model, AR Q tract length influences progression and castration response. Mutation profiling in mice provides direct evidence that somatic AR variants are selected by therapy, a finding validated in human metastases from distinct treatment groups. Mutant ARs exploit multiple mechanisms to resist hormone ablation, including alterations in ligand specificity, target gene selectivity, chaperone interaction and nuclear localization. Regardless of their frequency, these variants permute normal function to reveal novel means to target wild type AR and its key interacting partners.

Keywords: androgen receptor, polyglutamine tract, AR gain-of-function mutations, humanized mice, mouse prostate cancer models, therapy selection

1. Introduction

The androgen receptor (AR) is a crucial regulator of male physiology and orchestrates the development and function of the prostate gland. Cancer of the prostate is a major health problem, confounded in etiology by numerous genetic and environmental factors (Nelson et al., 2003). Despite the marked complexity and heterogeneity in the origin and course of prostate cancer, early stages of tumor growth depend on androgen and, despite therapies aimed at depleting hormone and blocking receptor transactivation, recurrent disease depends on reactivation of AR (Scher and Sawyers, 2005). Just as cancer cells evolve over time due to both intrinsic and extrinsic forces, so AR function transitions from maintaining homeostasis to promoting oncogenesis (Litvinov et al., 2003). Given AR's key role in these processes, variation in the structure or expression of the *Ar* gene is logically suspected of influencing initiation or progression of disease. Extensive investigation of *Ar* germline polymorphism and somatic mutation has produced inconsistent results on the importance of AR genetic variation in prostate cancer. Despite the controversies, many insights into wild type receptor function have been derived from analysis of genetic variants implicated in disease.

In order to use experimental tools to test the role of AR variants in prostate cancer, and to avoid the confounding heterogeneity of man, we established a mouse model in which the murine *Ar* gene was converted to the human sequence by germline recombination (Albertelli et al., 2006). The DNA and ligand binding domains (DBD, LBD) of human and mouse ARs are identical, with a few amino acid differences in the hinge region (Fig. 1). However the large amino termini (NTD) encompassing the major transactivation domains differ by 15% in protein sequence as well as in position and extent of polymorphic polyglutamine (Q) and polyglycine (G) tracts. Most of the NTD is encompassed within *Ar's* large first exon, allowing mouse and human sequences to be swapped by knock-in technology resulting in a locus in which hAR is expressed under murine regulatory control. This "humanized" model allows us to assess effects

of germline polymorphisms on disease phenotypes (Albertelli et al., 2008) and to evaluate relationships between somatic mutation and treatment regimen (O'Mahony et al., 2008). Comparison to patient data directly substantiates the relevance (Steinkamp et al., 2009).

Here we present findings pertaining to controversies in the role of the AR polyQ tract and *Ar* gene mutations in prostate cancer. We summarize recent key studies in the literature but focus primarily on results from our laboratory, including data from both mouse and human prostate cancer patients. More comprehensive reviews and discussion of implicated mechanisms have recently been published (Buchanan et al., 2009; Robins, 2009).

2. AR polymorphisms and prostate cancer

AR's NTD coordinates interactions with coregulators and nonreceptor transcription factors that are critical for both ligand-dependent and –independent transactivation (Robins, 2004; Shen and Coetzee, 2005). The N-terminal polyQ tract, encoded by a CAG repeat, was first linked to pathology in Kennedy disease. Abnormal expansion of this tract underlies late-onset atrophy of spinal and bulbar motor neurons due to misfolding and aggregation of the mutant AR, compounded by partial loss of function (Lieberman and Robins, 2008). Transfection assays ascribe the latter to an inverse correlation between Q tract length and AR transcriptional strength (Mhatre et al., 1993). Sensitivity to Q tract length difference within a "normal" range (9 – 37 CAGs) is likely due to effects on overall AR structure rather than to site-specific interactions (Irvine et al., 2000). Shorter Q tracts enhance AR's critical intramolecular N-C interaction, allowing response to lower androgen concentrations and more effective coactivation by p160 proteins (Buchanan et al., 2004; Wang et al., 2004). Because Q tract length affects AR activity in transfection, associations between such variation and male traits have been sought. Substantial evidence links AR Q tract length to male fertility (Davis-Dao et al., 2007) and in hypogonadal men to response to testosterone replacement (Zitzmann et al., 2004). Association

of Q tract length with prostate cancer, where a more active AR might promote oncogenesis, remains controversial.

2.1. Human genetic and genomic studies of the CAG repeat and prostate cancer

That short Q tract ARs increase prostate cancer risk was first conjectured based on their greater prevalence in the high-risk African-America population (Coetzee and Ross, 1994), and gained credence from their increased transcriptional activity *in vitro* and evidence of Q tract contraction in malignant but not benign prostate cells (Schoenberg et al., 1994; Alvarado et al., 2005). Epidemiological studies initially found association between fewer CAG repeats and increased risk or more aggressive disease (e.g., (Irvine et al., 1995; Giovannucci et al., 1997; Stanford et al., 1997)). However results vary with ethnic group and most recent studies find no association with risk (e.g., (Lindstrom et al., 2010; Price et al., 2010)). Conflicting conclusions stem from many aspects of study design, including small sample sizes, differences in environment, genetic admixture, ascertainment bias (including diagnosis before or after the age of PSA testing) and differences in tract length cut-off points. Regardless of these variables, modern genotyping technology applied to large case-controlled groups fails to substantiate *Ar* CAG tract length as a predictor of prostate cancer (Price et al., 2010). *Ar* may be more comparable to a quantitative trait, the effect of which depends on variation at other loci and on environmental interactions. In support of this, allelic differences in genes participating with *Ar* in the androgen axis, including those encoding enzymes of testosterone synthesis (cytochrome P450c17) and conversion (steroid-5-α-reductase type 2), reveal haplotypes that do differ significantly in prostate cancer risk (Lindstrom et al., 2006).

While *Ar*'s CAG repeat may not impact risk of prostate cancer, it may affect other aspects of disease that are subject to AR function, such as progression or treatment response. Several studies support a relationship between Q tract length and serum levels of testosterone and estradiol, likely due to the effect of differential AR strength in systemic feedback control

(Huhtaniemi et al., 2009; Lindstrom et al., 2010). Whether Q tract variation might also impact intratumoral hormone levels, or their effect, is of interest in light of increasing evidence of prostatic androgen synthesis (Montgomery et al., 2008). Furthermore, CAG repeats are inherently unstable and Q tract contraction is found in malignant prostate cells but not in adjacent normal tissue (Alvarado et al., 2005). Heterogeneous somatic variation in Q tract length may play a role in the increased AR activity that occurs in prostate cancer recurrence. Given the complex and contradictory data on the role of the Q tract in prostate cancer, we turned to a mouse model to gain biological insight.

2.2. Effects of polyQ tract length on AR biology: insights from genetically engineered mice

To investigate the effect of AR Q tract length on prostate cancer, we developed knock-in strains with *hAr* alleles containing 12, 21 or 48 CAG repeats (Albertelli et al., 2006). All mice in this allelic series (referred to as AR12Q, AR21Q, or AR48Q mice) are grossly normal and have similar fertility, with no neurological problems evident in the AR48Q mice (much longer CAG repeats are needed to model neurodegeneration) (Lieberman and Robins, 2008). Transactivational differences due to Q tract length are however detectable in expression of prostatic AR target genes. Moreover, allele-dependent responses following hormonal perturbation corroborate a role of AR Q tract length in systemic androgen sensitivity (*ms. in press*). Therefore these mice model human AR Q tract length variation within a “normal” range.

Introducing a prostate-targeted SV40 T-antigen (Tag) oncogene by crossing to TRAMP mice reveals differential effects of the humanized *Ar* alleles on cancer initiation, progression and response to androgen ablation (Albertelli et al., 2008; Robins et al., 2008) (Fig. 2). Mice with short Q tract ARs develop prostatic intraepithelial neoplasia (PIN) earlier than mice with median (21Q) or long (48Q) tracts. However, the resulting AR12Q tumors are well differentiated, progress slowly and allow longer survival of AR12Q than AR21Q mice. Remarkably, following androgen ablation Q tract length effects are in directions opposite to those in hormone intact

mice. Castrated AR12Q mice develop tumors later than the other strains and live longer than their intact littermates, indicating good response to treatment. In contrast, AR48Q-TRAMP mice fail to benefit from androgen ablation. Detection of these divergent responses due to a modest variation in AR activity is more evident on a homogeneous genetic background. This suggests that *Ar* variation at the extremes of the normal range may also impact certain cases of human disease.

Effects in TRAMP mice are complicated by the AR dependence of the oncogenic transgene, but that is unlikely to account for all differences noted. In some regards this complexity mimics the androgen-responsiveness of *TMPRSS2-ERG* fusion genes that are critical in progression (Tomlins et al., 2007). Some of the tumor characteristics may reflect varying AR functions at different disease stages and in distinct cell types (e.g., stroma vs. epithelia). For example, it is reasonable to predict that weak AR action in TRAMP would enhance castration response due to loss of the androgen-driven Tag oncogene. Instead, AR48Q castrates fare worse after castration, perhaps because weak stromal AR fails to repress growth of pre-existing androgen-independent cells. Similarly, the hypersensitive AR12Q may more ably maintain epithelial differentiation despite hormone ablation and thereby slow tumor growth. Results from these mice supports further investigation of an influence of AR Q tract variation in later stage human disease. In addition these mice provide a unique experimental model in which to define downstream events that predict response to therapy. Our future studies will elucidate mechanisms by which Q tract length impacts ligand-independent AR function and define corresponding pathophysiology in models that more accurately reflect human disease.

3. AR alterations in prostate cancer

Somatic mutation is a hallmark of cancer, whereby uncontrolled growth stems from loss of tumor suppressor genes and aberrant function of oncogenes. Coincident genomic instability

leads to a mutator phenotype, in which each cancer cell may come to harbor hundreds of mutations (Bielas et al., 2006). Most mutations likely have little phenotypic effect and are considered “passengers”, but some may be “drivers” that provide a growth advantage or promote metastasis (Greenman et al., 2007; Jones et al., 2008). Recent studies using genomic tools provide quantitative data reinforcing this view. A high-throughput interrogation of 1,000 tumors for known mutations indicates that very few mutations occur frequently but mutations in several oncogenes co-occur (e.g., *PIK3CA* mutations coincide with mutations in *KRAS*), highlighting sensitive pathways rather than individual genes as potential therapeutic targets (Thomas et al., 2007). Genome-wide characterization shows that mutation rates and genes mutated vary substantially across tumor types and subtypes (Kan et al., 2010). Interestingly, mutation rates appear relatively low in prostate cancer, where ~75% of tumors carry *TMPRSS2-ERG* gene fusions (Tomlins et al., 2007). In contrast, in a lung tumor next-generation sequencing revealed a wide variety of somatic mutations, with differing patterns in expressed vs. nonexpressed genes (Lee et al., 2010). While most of these mutations are likely passengers some, such as those occurring in kinase genes, appear to be drivers because they evidence distinct selective pressures within the tumor environment. For prostate cancer, numerous mutations have been identified in *Ar* and many are gain of function (Buchanan et al., 2009). However, most aberrant ARs have been identified in small studies and the effects of these mutations have largely been determined *in vitro* (a notable exception is AR-E231G – see below). Therefore *Ar* mutation frequency and the functional significance of mutant ARs in disease remain unclear. As with the Q tract controversy, genomic approaches and mouse models are producing new insights.

3.1 AR mutations – driving in heavy traffic

Somatic gain-of-function mutations in AR are compelling because their actions implicate mechanisms of disease recurrence in castration resistant prostate cancer (CRPC). Mutations

have been identified in tumors that alter ligand specificity, increase sensitivity to low androgen levels or allow ligand-independent activation, all proposed as mechanisms of therapy resistance (Feldman and Feldman, 2001). The paradigm of a mutant that could evade treatment is AR-T877A, first identified in the LNCaP cell line and subsequently in several advanced prostate cancers (Taplin et al., 1995). The subtle shift in LBD structure due to T877A permits various noncanonical ligands, including the antiandrogen hydroxyflutamide, to confer an active conformation (Hur et al., 2004). Other LBD mutations that result in promiscuous ARs, such as H875Y, also harbor clear potential for hormone resistance. Mutations in the NTD could lead to androgen-independent AR activation but fewer examples have been identified since sequencing this region is impeded by its high GC content and polymorphic repeats. Moreover, most studies have examined small numbers of patients using methods that do not detect low frequency *Ar* mutations, thus underestimating the heterogeneity of prostate cancer between individuals as well as within the tumor. Nevertheless, *Ar* mutations have been reported in up to one-third of tumor samples and most commonly following treatment, suggesting they may be selected by therapy (Taplin et al., 1999). The use of microdissection and laser capture has greatly improved the ability to detect *Ar* mutations (Marcelli et al., 2000; Alvarado et al., 2005).

Recent comprehensive surveys with large sample sizes support the common occurrence of somatic AR alterations in prostate cancer progression, with the most frequent event being gene amplification (Holcomb et al., 2009; Liu et al., 2009; Taylor et al., 2010; Robbins et al., 2011). Targeted exon sequencing of genes of high interest to prostate cancer identified promiscuous AR mutants, including AR-T877A and AR-H875Y, in 5-10% of the samples (Taylor et al., 2010; Robbins et al., 2011). The methodology however would not have detected variants present in a subpopulation of cells or prematurely truncated ARs due to nonsense mutation or alternative splicing. The latter has become a focus of recent attention (Jagla et al., 2007; Dehm et al., 2008; Hu et al., 2009). There is surprising diversity in these splice variants, many of which lack C-terminal sequences and thereby could encode constitutive ARs that drive

castration resistance. However, most variants in tumor cells are low in abundance relative to correctly processed *Ar*, appear in response to hormone withdrawal and some may require wild type receptor to function, making it unclear whether they play a dominant role in resistance to therapy (Watson et al., 2010). Recently in a cell-based model, truncated ARs due to intragenic rearrangements have been shown to promote prostate cancer progression (Li et al., 2011). As with mutant ARs, further study of alternatively spliced forms may shed critical light on how wild type receptor drives progression despite antiandrogen therapy.

3.2. AR mutation profiling in prostate tumors of mice and men

In mice, genetic background, environment and treatment can be controlled, allowing better assessment of the prevalence of *Ar* mutations in prostate cancer and the extent to which variant ARs reflect tumor biology. Prior study of intact (untreated) versus castrated (androgen ablated) TRAMP mice found that missense *Ar* mutations in primary tumors vary with hormonal status (Han et al., 2001). To obtain more direct evidence that this is due to the selection pressure of therapy, we used the humanized AR mice to ask whether distinct types of mutations arise with different treatments. hAR21Q-TRAMP mice were either untreated, castrated at 12 weeks (when PIN but not overt cancer is present), or treated with different antiandrogens upon detection of a palpable tumor. Comparison of bicalutamide to flutamide is particularly informative – the bulkier bicalutamide more significantly distorts the AR LBD and so unlike flutamide is less likely to impose an agonist conformation from a single amino acid change (e.g., AR-T877A). To identify potentially functional AR mutants that might be present in only some cells, the equivalent of 20 *Ar* mRNAs per tumor were sequenced from reverse-transcribed, PCR-amplified and subcloned cDNAs (O'Mahony et al., 2008). *Ar* sequences from testes of these mice provide an estimate of technical error rate; sequence differences in tumor samples were twice as frequent as in testes. To highlight changes more likely to be actual mutations, only those that recurred within a tumor or occurred in multiple tumors were studied. Since only

one codon change recurred in testis this conservative view minimizes methodological errors. By this approach, missense mutations in *Ar* that repeat within or between tumors occur at a rate of ~0.5/10 Kbp, with most having an overall tumor frequency of less than 10% of the cells.

Recurring missense AR mutations are least frequent in tumors from untreated mice, evidencing effects of treatment and corroborating clinical observations (Taplin et al., 1999). Mutations following castration occur mostly in the NTD, whereas flutamide-treated mice have more mutations in the C-terminal portion, including some premature stop codons that could lead to constitutively active ARs. Moreover, flutamide-treated mice have the most treatment-specific mutations. Bicalutamide treatment, as expected, led to fewer mutations that primarily occurred in the NTD. Many of the AR mutations in mouse prostate tumors cluster to regions found previously mutated in patients and are generally distinct from loss-of-function mutations identified in Androgen Insensitivity Syndrome (AIS) (Buchanan et al., 2009). Many mutations cluster near the most highly conserved segment of the LBD that affects ligand specificity (Fenton et al., 1997). Some of these that are known to be loss of function as germline mutations are surprisingly active in transfection assays. This suggests that mutations that are null in development may display partial or altered function in other contexts, such as the tumor environment (see below).

To compare directly in man whether mutations differ in number or type after treatment, and whether distinct antagonists select for different mutations, we performed a similar analysis with a set of high quality samples of metastases from either hormone-naïve patients or those treated with only one antagonist (flutamide or bicalutamide) (Steinkamp et al., 2009). As with the mouse samples, the equivalent of 20 *Ar* mRNAs were sequenced and similar base change rates were found. Interestingly, despite having fewer human than mouse tumors, more mutations recurred at higher rates (both in more tumors and at higher levels per tumor), providing stronger evidence of treatment selection. In general, mutations occurring at low frequency but in multiple tumors are located in the NTD. In contrast, mutations present in

several clones from a single patient tend to be case-specific, not restricted by AR domain and reflective of treatment differences (Fig. 3). Few mutations recur in hormone-naïve samples. The "mutator phenotype" is not equal between samples, with less than half of the tumors accounting for most of the mutations.

Several similarities emerge from our mouse and human *Ar* mutation profiling, including comparable overall mutation frequencies and fewer mutations in hormone-naïve tumors. The variety of recurring mutations suggests that selective value may stem from numerous rare mutations rather than a few common ones, with detection of rare events dependent on sequencing methodology. A significant difference between the mouse and human studies is that more mutations were present at higher frequencies in man. This may reflect the longer time with disease in man allowing a greater period for selection to operate (Steinkamp et al., 2009). Furthermore, human metastases are more clonal in nature than heterogeneous mouse primary tumors. In fact, recent analyses using next generation sequencing suggest somatic genetic variation and selection play a greater role in cancer ontogeny than previously suspected (Gottlieb et al., 2010). Even silent base changes are not random, suggesting selection for greater AR activity may operate at the level of efficiency of translation or co-translational protein folding. Below specific examples illustrate how *Ar* mutations may be functionally relevant to prostate cancer progression.

3.3 Mutant ARs exploit multiple mechanisms to evade therapy

Mutations found in numerous studies cumulatively highlight AR domains within which different mutations may affect a common function and produce a similar phenotype (Buchanan et al., 2009). Further evidence that these mutant ARs may not be simply bystanders is inherent in the means they may use to evade therapy and is reinforced by finding fewer additional mutations in tumors with prevalent variant ARs. For example, the human tumor in which we identified the promiscuous AR-V716M (below) had only one other missense mutation

(Steinkamp et al., 2009). Similarly, few AR mutations recur in xenograft tumors originating from VCaP cells that have amplified *Ar* – additional variation may have little value when high levels of wild type AR are attained (*O'Mahony and Robins, unpublished*).

3.3.1 Promiscuity is priceless: AR-V716M

A mutation most clearly supporting treatment selection is V716M, an alteration known to create a permissive receptor responsive to a wide array of hormones and antagonists (Culig et al., 1993). This mutation was present in all 20 *Ar* clones sequenced from the lung metastasis of a flutamide-treated patient whose normal tissue DNA was wild type (Steinkamp et al., 2009). Two additional metastases from this patient also yielded only the mutant sequence, indicating a clonal population carrying AR-V716M accounted for these three metastases and therefore arose either early in metastatic invasion or within the primary tumor itself. The absence of other *Ar* mutations recurring in this patient suggests this variant was effective enough to reduce selective value of other mutations. While fixation of a mutation like V716M may be relatively rare, many cancers may have subsets of cells with different mutations that together provide a similar growth advantage. Interestingly, the patient with AR-V716M survived 19 years beyond diagnosis. It is tempting to speculate that in this case residual activity of the promiscuous AR fostered slower tumor growth than a poorly differentiated tumor lacking AR altogether.

3.3.2 Unchaperoned oncogenic behavior: AR-E255K

The AR NTD has been less examined for somatic mutation, but in the eleven human samples we studied 19 mutations recurred in the NTD and 14 of these fell into known functional motifs. The most highly conserved region of the AR NTD encodes a domain that interacts with CHIP (**C**OOH-terminus of **H**SP70-**I**nteracting **P**rotein), an E3-ubiquitin ligase that controls steady-state levels of AR by promoting its degradation (He et al., 2004). *Ar* mutations in this region have been identified in both mouse and human prostate tumors and may abrogate cell

checks on AR activity. Importantly, the mouse mutant, AR-E231G, is sufficient for oncogenesis as a prostate-targeted transgene (Han et al., 2005). A similar human mutant, AR-E255K, from a flutamide-treated patient prolongs the half-life of AR in both the presence and absence of ligand (Steinkamp et al., 2009). Moreover, a substantial proportion of AR-E255K localizes to the nucleus in the absence of hormone. CHIP interaction domain mutants are not only unfettered from ligand control but also exhibit increased and differential transactivation (Buchanan et al., 2009; Steinkamp et al., 2009). For AR-E255K this is more notable in benign RWPE cells than in malignant PC-3 cells and varies with target promoter; differences in specificity as well as activity could reflect differences in the array of host coregulators. Varied transactivation by mutant ARs demonstrate how they not only drive persistent gene expression in the face of androgen ablation but that they may do so differentially, promoting distinct pathways of tumor progression that may differ in selective value dependent on disease stage.

3.3.3 Avenues of differential gene expression: AR-W435L, AR-R753Q

Mutations in almost any receptor domain may influence target gene selection via conformational changes that impact recognition or affinity for the response element or that alter intra- or intermolecular interactions. Two mutations from our studies exemplify the multiple ways transcriptional selectivity may be affected. AR-W435L, found in two antiandrogen-treated patients, alters the WxxLF motif that plays a role in AR's critical N-C interaction. While this motif is secondary to the more N-terminal FxxLF motif essential for ligand-dependent activity, WxxLF may be more important in ligand-independent activation (Dehm et al., 2007). W435L changes the motif to LxxLF, which bears greater similarity to the LxxLL interaction motif of coactivators. The effect of this is to strengthen N-C interaction, as demonostrated in a mammalian two-hybrid assay, and to confer stronger transactivation of a reporter gene (Steinkamp et al., 2009). Interestingly, AR-W435L activity varies with the host cell and target promoter tested, suggesting

differential promoter recognition as well as broadly increased AR activity may have selective value and may change over the course of disease.

Some AR alterations that arise in prostate cancer are presumed to be null mutations, particularly if they were previously identified in AIS. Yet some have partial or altered function that could be significant in cancer. AR-R753Q is especially intriguing since as a germline mutation it underlies rat testicular feminization as well as cases of human AIS. The molecular basis for this is reduced androgen binding capacity resulting in impaired N-C interaction. In transfection into prostate cells, AR-R753Q proves to be as potent as wild type AR at activating a subset of promoters. AR-R753Q fails to activate selective AREs that bind AR with greater specificity but show weak activation (O'Mahony et al., 2008). A similar inability to activate AR selective elements occurs in a mouse with an AR DBD mutation that leads to reduced fertility (Schauwaers et al., 2007). Thus although germline AR-R753Q cannot activate genes critical for male development, somatic AR-R753Q may induce a subset of genes, perhaps those involved in oncogenic rather than differentiative pathways. Numerous other mutations may produce a similar loss of function in the context of development but a gain of function in the context of cancer.

3.3.4 Alternative splicing as a path of resistance: AR23

Variant proteins that arise from alternative splicing (distinct from actual mutation) are widespread in cancer (David and Manley, 2010) and may be particularly significant in CRPC where constitutive ARs can be produced from misspliced transcripts that omit the LBD (Dehm et al., 2008; Hu et al., 2009). The mechanism by which these variants arise is unclear, but the fact that their occurrence increases rapidly when androgen is withdrawn and recedes when hormone is readministered suggests AR may be involved, directly or indirectly, in processing its own transcript. AR, like other steroid receptors, is known to participate in splicing (Auboeuf et al., 2002; Dong et al., 2007) and disease conditions or aberrant ARs may foster aberrant splicing

events (Yu et al., 2009). Many misspliced ARs terminate prematurely, destroying their ability to bind ligand and freeing them from cytoplasmic tethering by HSP90, thus permitting their localization to the nucleus where they are constitutively active. These variant forms are usually a small fraction of the total *Ar* mRNA in the cell and it remains unclear whether they provide a major means of treatment resistance (Watson et al., 2010; Li et al., 2011).

A distinct misspliced *Ar* called AR23 differs from the constitutive variants in lacking intrinsic activity but promoting full-length AR function. We found this variant in the majority of antiandrogen-treated patient samples (Steinkamp et al., 2009). Use of a cryptic splice site in intron 2 inserts 69 bps in frame to create a 23 amino acid extension between the two zinc fingers of the DBD. AR23 was previously identified in an AIS patient due to a mutation upstream of exon 3 and also in a bicalutamide-treated prostate cancer patient (Bruggenwirth et al., 1997; Jagla et al., 2007). Beyond being incapable of binding DNA, AR23 forms cytoplasmic speckles in response to hormone. While this receptor cannot participate directly in gene regulation, cotransfection of AR23 with wild type AR leads to greater activation of a reporter gene, even in the presence of antagonist. This action is not specific to AR since some other transcription factors are also enhanced in their activity in the presence of AR23. AR23 likely exists in an unfolded state, as suggested by its cytoplasmic aggregation, which may compromise the cell chaperone system critical for normal protein activity. In this manner AR23 may sustain wild type AR activity in the presence of antagonists, as supported by its prevalence in treated but not untreated patients.

4. Concluding Remarks

The genomic era has added significant depth to our understanding of prostate cancer. Some discoveries are remarkable, such as the unanticipated and broad role of *TMPRRS2-ERG* gene fusions. These findings only serve to substantiate AR as a central player in all stages of disease and, as yet, the key target in therapy. Studies with genetically engineered mice allow

functional analyses not possible in man, using homogeneous genetic backgrounds and uniform environments to highlight distinct disease modalities. Results from our lab and others confirm the relevance of *Ar* genetic variation to disease progression, and derive novel insights into mechanisms of treatment resistance from specific variations.

In mice, differences in AR Q tract length exert measurable effects on disease parameters including development of PIN and rate of cancer progression, both in the presence and absence of hormone. While Q tract length is not by itself a risk factor for prostate cancer in man, it contributes to variation in the androgen axis, in which many genes conspire to influence hormone sensitivity. Q tract lengths at the extremes of the normal range may have more detectable effects when hormone levels are changing, as occurs in development and aging, or clinically, as when hormone is replaced in hypogonadal men or ablated in prostate cancer treatment. Individual variation in efficacy of new drugs that more effectively inhibit androgen synthesis (e.g., abiraterone) may reveal an influence of AR Q tract length.

Somatic *Ar* mutations are generally low in frequency but provide valuable information about mechanisms of treatment resistance. Myriad mutations affect multiple distinct functions (specificity of hormone and DNA binding, coactivator and chaperone interactions, nuclear localization), with downstream gene expression programs varying with the receptor form. AR's enormous plasticity in evading treatment is similar to the complexity p53 demonstrates whereby many different mutations drive transition from tumor suppressor to oncogene. The mutability of AR is but one factor making it unlikely that single agents will universally abolish activity. Promising new drugs such as MDV3100 and EPI-001 provide new environments for selection (Sadar, 2011); cases of resistance may reveal additional novel AR alterations. Moreover, improved anti-AR therapies may impair differentiation and therefore be ineffective in redirecting cell growth in certain instances. The mutant ARs direct attention to upstream and downstream interacting partners that may provide new ways to combat disease in general or to fight

resistance in specific cases. The broad landscape of low level *Ar* mutation suggests AR is more than a passenger, but not often a driver. Nevertheless the difficulty of silencing AR in prostate cancer suggests it acts as a backseat driver.

Acknowledgements

This review summarizes work by members of the laboratory, particularly highlighting past contributions of Mara Steinkamp, Orla O'Mahony, Megan Albertelli and Arno Scheller. Beth LaPensee and Chris Krebs provided valuable discussions. Work was supported by the NIH (NIDDK-RO1-56356, NCI-P50-CA69568, NCI-RO1-144032) and the DOD (DOD17-02-1-0099, W81XWH-05-1-0105, W81XWH-08-1-0034), as well as Core services of the University of Michigan Cancer Center (5 P30 CA46592) and the Michigan Diabetes Research and Training Center (5 P60 DK20572).

References

Albertelli, M.A., O'Mahony, O.A., Brogley, M., Tosoian, J., Steinkamp, M., Daignault, S., Wojno, K., Robins, D.M., 2008. Glutamine tract length of human androgen receptors affects hormone-dependent and -independent prostate cancer in mice. Hum Mol Genet 17: 98-110.

Albertelli, M.A., Scheller, A., Brogley, M., Robins, D.M., 2006. Replacing the mouse androgen receptor with human alleles demonstrates glutamine tract length-dependent effects on physiology and tumorigenesis in mice. Mol Endocrinol 20: 1248-60.

Alvarado, C., Beitel, L.K., Sircar, K., Aprikian, A., Trifiro, M., Gottlieb, B., 2005. Somatic mosaicism and cancer: a micro-genetic examination into the role of the androgen receptor gene in prostate cancer. Cancer Res 65: 8514-8.

Auboeuf, D., Honig, A., Berget, S.M., O'Malley, B.W., 2002. Coordinate regulation of transcription and splicing by steroid receptor coregulators. Science 298: 416-9.

Bielas, J.H., Loeb, K.R., Rubin, B.P., True, L.D., Loeb, L.A., 2006. Human cancers express a mutator phenotype. Proc Natl Acad Sci U S A 103: 18238-42.

Bruggenwirth, H.T., Boehmer, A.L., Ramnarain, S., Verleun-Mooijman, M.C., Satijn, D.P., Trapman, J., Grootegoed, J.A., Brinkmann, A.O., 1997. Molecular analysis of the androgen-receptor gene in a family with receptor-positive partial androgen insensitivity: an unusual type of intronic mutation. Am J Hum Genet 61: 1067-77.

Buchanan, G., Need, E.F., Bianco-Miotto, T., Greenberg, N.M., Scher, H., Centenera, M.M., Butler, L.M., Robins, D.M., Tilley, W.D. (2009). Insights from AR Gene Mutations. Androgen Action in Prostate Cancer. D. Tindall and J. Mohler. New York, Springer**:** 207-240.

Buchanan, G., Yang, M., Cheong, A., Harris, J.M., Irvine, R.A., Lambert, P.F., Moore, N.L., Raynor, M., Neufing, P.J., Coetzee, G.A., Tilley, W.D., 2004. Structural and functional consequences of glutamine tract variation in the androgen receptor. Hum Mol Genet 13: 1677-92.

Coetzee, G.A., Ross, R.K., 1994. Re: Prostate Cancer and the Androgen Receptor. Journal of the National Cancer Institute 86: 872-873.

Culig, Z., Hobisch, A., Cronauer, M.V., Cato, A.C., Hittmair, A., Radmayr, C., Eberle, J., Bartsch, G., Klocker, H., 1993. Mutant androgen receptor detected in an advanced-stage prostatic carcinoma is activated by adrenal androgens and progesterone. Mol Endocrinol 7: 1541-50.

David, C.J., Manley, J.L., 2010. Alternative pre-mRNA splicing regulation in cancer: pathways and programs unhinged. Genes Dev 24: 2343-64.

Davis-Dao, C.A., Tuazon, E.D., Sokol, R.Z., Cortessis, V.K., 2007. Male Infertility and Variation in CAG Repeat Length in the Androgen Receptor Gene: A Meta-analysis. J Clin Endocrinol Metab 92: 4319-26.

Dehm, S.M., Regan, K.M., Schmidt, L.J., Tindall, D.J., 2007. Selective role of an NH2-terminal WxxLF motif for aberrant androgen receptor activation in androgen depletion independent prostate cancer cells. Cancer Res 67: 10067-77.

Dehm, S.M., Schmidt, L.J., Heemers, H.V., Vessella, R.L., Tindall, D.J., 2008. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. Cancer Res 68: 5469-77.

Dong, X., Sweet, J., Challis, J.R., Brown, T., Lye, S.J., 2007. Transcriptional activity of androgen receptor is modulated by two RNA splicing factors, PSF and p54nrb. Mol Cell Biol 27: 4863-75.

Feldman, B.J., Feldman, D., 2001. The development of androgen-independent prostate cancer. Nat Rev Cancer 1: 34-45.

Fenton, M.A., Shuster, T.D., Fertig, A.M., Taplin, M.E., Kolvenbag, G., Bubley, G.J., Balk, S.P., 1997. Functional characterization of mutant androgen receptors from androgen-independent prostate cancer. Clin Cancer Res 3: 1383-8.

Giovannucci, E., Stampfer, M.J., Krithivas, K., Brown, M., Dahl, D., Brufsky, A., Talcott, J., Hennekens, C.H., Kantoff, P.W., 1997. The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. Proc Natl Acad Sci U S A 94: 3320-3.

Gottlieb, B., Beitel, L.K., Alvarado, C., Trifiro, M.A., 2010. Selection and mutation in the "new" genetics: an emerging hypothesis. Hum Genet 127: 491-501.

Greenman, C., Stephens, P., Smith, R., Dalgliesh, G.L., Hunter, C., Bignell, G., Davies, H., Teague, J., Butler, A., Stevens, C., Edkins, S., O'Meara, S., Vastrik, I., Schmidt, E.E., Avis, T., Barthorpe, S., Bhamra, G., Buck, G., Choudhury, B., Clements, J., Cole, J., Dicks, E., Forbes, S., Gray, K., Halliday, K., Harrison, R., Hills, K., Hinton, J., Jenkinson, A., Jones, D., Menzies, A., Mironenko, T., Perry, J., Raine, K., Richardson, D., Shepherd, R., Small, A., Tofts, C., Varian, J., Webb, T., West, S., Widaa, S., Yates, A., Cahill, D.P., Louis, D.N., Goldstraw, P., Nicholson, A.G., Brasseur, F., Looijenga, L., Weber, B.L., Chiew, Y.E., DeFazio, A., Greaves, M.F., Green, A.R., Campbell, P., Birney, E., Easton, D.F., Chenevix-Trench, G., Tan, M.H., Khoo, S.K., Teh, B.T., Yuen, S.T., Leung, S.Y., Wooster, R., Futreal, P.A., Stratton, M.R., 2007. Patterns of somatic mutation in human cancer genomes. Nature 446: 153-8.

Han, G., Buchanan, G., Ittmann, M., Harris, J.M., Yu, X., Demayo, F.J., Tilley, W., Greenberg, N.M., 2005. Mutation of the androgen receptor causes oncogenic transformation of the prostate. Proc Natl Acad Sci U S A 102: 1151-6.

Han, G., Foster, B.A., Mistry, S., Buchanan, G., Harris, J.M., Tilley, W.D., Greenberg, N.M., 2001. Hormone status selects for spontaneous somatic androgen receptor variants that demonstrate specific ligand and cofactor dependent activities in autochthonous prostate cancer. J Biol Chem 276: 11204-13.

He, B., Bai, S., Hnat, A.T., Kalman, R.I., Minges, J.T., Patterson, C., Wilson, E.M., 2004. An androgen receptor NH2-terminal conserved motif interacts with the COOH terminus of the Hsp70-interacting protein (CHIP). J Biol Chem 279: 30643-53.

Holcomb, I.N., Young, J.M., Coleman, I.M., Salari, K., Grove, D.I., Hsu, L., True, L.D., Roudier, M.P., Morrissey, C.M., Higano, C.S., Nelson, P.S., Vessella, R.L., Trask, B.J., 2009. Comparative analyses of chromosome alterations in soft-tissue metastases within and across patients with castration-resistant prostate cancer. Cancer Res 69: 7793-802.

Hu, R., Dunn, T.A., Wei, S., Isharwal, S., Veltri, R.W., Humphreys, E., Han, M., Partin, A.W., Vessella, R.L., Isaacs, W.B., Bova, G.S., Luo, J., 2009. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res 69: 16-22.

Huhtaniemi, I.T., Pye, S.R., Limer, K.L., Thomson, W., O'Neill, T.W., Platt, H., Payne, D., John, S.L., Jiang, M., Boonen, S., Borghs, H., Vanderschueren, D., Adams, J.E., Ward, K.A., Bartfai, G., Casanueva, F., Finn, J.D., Forti, G., Giwercman, A., Han, T.S., Kula, K., Lean, M.E., Pendleton, N., Punab, M., Silman, A.J., Wu, F.C., 2009. Increased estrogen rather than decreased androgen action is associated with longer androgen receptor CAG repeats. J Clin Endocrinol Metab 94: 277-84.

Hur, E., Pfaff, S.J., Payne, E.S., Gron, H., Buehrer, B.M., Fletterick, R.J., 2004. Recognition and accommodation at the androgen receptor coactivator binding interface. PLoS Biol 2: E274.

Irvine, R.A., Ma, H., Yu, M.C., Ross, R.K., Stallcup, M.R., Coetzee, G.A., 2000. Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. Hum Mol Genet 9: 267-74.

Irvine, R.A., Yu, M.C., Ross, R.K., Coetzee, G.A., 1995. The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. Cancer Res 55: 1937-40.

Jagla, M., Feve, M., Kessler, P., Lapouge, G., Erdmann, E., Serra, S., Bergerat, J.P., Ceraline, J., 2007. A splicing variant of the androgen receptor detected in a metastatic prostate cancer exhibits exclusively cytoplasmic actions. Endocrinology 148: 4334-43.

Jones, S., Chen, W.D., Parmigiani, G., Diehl, F., Beerenwinkel, N., Antal, T., Traulsen, A., Nowak, M.A., Siegel, C., Velculescu, V.E., Kinzler, K.W., Vogelstein, B., Willis, J., Markowitz, S.D., 2008. Comparative lesion sequencing provides insights into tumor evolution. Proc Natl Acad Sci U S A 105: 4283-8.

Kan, Z., Jaiswal, B.S., Stinson, J., Janakiraman, V., Bhatt, D., Stern, H.M., Yue, P., Haverty, P.M., Bourgon, R., Zheng, J., Moorhead, M., Chaudhuri, S., Tomsho, L.P., Peters, B.A., Pujara, K., Cordes, S., Davis, D.P., Carlton, V.E., Yuan, W., Li, L., Wang, W., Eigenbrot, C., Kaminker, J.S., Eberhard, D.A., Waring, P., Schuster, S.C., Modrusan, Z., Zhang, Z., Stokoe, D., de Sauvage, F.J., Faham, M., Seshagiri, S., 2010. Diverse somatic mutation patterns and pathway alterations in human cancers. Nature 466: 869-73.

Lee, W., Jiang, Z., Liu, J., Haverty, P.M., Guan, Y., Stinson, J., Yue, P., Zhang, Y., Pant, K.P., Bhatt, D., Ha, C., Johnson, S., Kennemer, M.I., Mohan, S., Nazarenko, I., Watanabe, C., Sparks, A.B., Shames, D.S., Gentleman, R., de Sauvage, F.J., Stern, H., Pandita, A., Ballinger, D.G., Drmanac, R., Modrusan, Z., Seshagiri, S., Zhang, Z., 2010. The mutation spectrum revealed by paired genome sequences from a lung cancer patient. Nature 465: 473-7.

Li, Y., Alsagabi, M., Fan, D., Bova, G.S., Tewfik, A.H., Dehm, S.M., 2011. Intragenic rearrangement and altered RNA splicing of the androgen receptor in a cell-based model of prostate cancer progression. Cancer Res 71: 2108-17.

Lieberman, A.P., Robins, D.M., 2008. The androgen receptor's CAG/glutamine tract in mouse models of neurological disease and cancer. J Alzheimers Disease 14: 247-55.

Lindstrom, S., Ma, J., Altshuler, D., Giovannucci, E., Riboli, E., Albanes, D., Allen, N.E., Berndt, S.I., Boeing, H., Bueno-de-Mesquita, H.B., Chanock, S.J., Dunning, A.M., Feigelson, H.S., Gaziano, J.M., Haiman, C.A., Hayes, R.B., Henderson, B.E., Hunter, D.J., Kaaks, R., Kolonel, L.N., Le Marchand, L., Martinez, C., Overvad, K., Siddiq, A., Stampfer, M., Stattin, P., Stram, D.O., Thun, M.J., Trichopoulos, D., Tumino, R., Virtamo, J., Weinstein, S.J., Yeager, M., Kraft, P., Freedman, M.L., 2010. A large study of androgen receptor germline variants and their relation to sex hormone levels and prostate cancer risk. Results from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium. J Clin Endocrinol Metab 95: E121-7.

Lindstrom, S., Wiklund, F., Adami, H.O., Balter, K.A., Adolfsson, J., Gronberg, H., 2006. Germline genetic variation in the key androgen-regulating genes androgen receptor, cytochrome P450, and steroid-5-alpha-reductase type 2 is important for prostate cancer development. Cancer Res 66: 11077-83.

Litvinov, I.V., De Marzo, A.M., Isaacs, J.T., 2003. Is the Achilles' heel for prostate cancer therapy a gain of function in androgen receptor signaling? J Clin Endocrinol Metab 88: 2972-82.

Liu, W., Laitinen, S., Khan, S., Vihinen, M., Kowalski, J., Yu, G., Chen, L., Ewing, C.M., Eisenberger, M.A., Carducci, M.A., Nelson, W.G., Yegnasubramanian, S., Luo, J., Wang, Y., Xu, J., Isaacs, W.B., Visakorpi, T., Bova, G.S., 2009. Copy number analysis indicates monoclonal origin of lethal metastatic prostate cancer. Nat Med 15: 559-65.

Marcelli, M., Ittmann, M., Mariani, S., Sutherland, R., Nigam, R., Murthy, L., Zhao, Y., DiConcini, D., Puxeddu, E., Esen, A., Eastham, J., Weigel, N.L., Lamb, D.J., 2000. Androgen receptor mutations in prostate cancer. Cancer Res 60: 944-9.

Mhatre, A.N., Trifiro, M.A., Kaufman, M., Kazemi-Esfarjani, P., Figlewicz, D., Rouleau, G., Pinsky, L., 1993. Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. Nat Genet 5: 184-8.

Montgomery, R.B., Mostaghel, E.A., Vessella, R., Hess, D.L., Kalhorn, T.F., Higano, C.S., True, L.D., Nelson, P.S., 2008. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res 68: 4447-54.

Nelson, W., DeMarzo, A., Isaacs, W., 2003. Mechanisms of Disease: Prostate Cancer. New England Journal of Medicine 349: 366-381.

O'Mahony, O.A., Steinkamp, M.P., Albertelli, M.A., Brogley, M., Rehman, H., Robins, D.M., 2008. Profiling human androgen receptor mutations reveals treatment effects in a mouse model of prostate cancer. Mol Cancer Res 6: 1691-701.

Price, D.K., Chau, C.H., Till, C., Goodman, P.J., Baum, C.E., Ockers, S.B., English, B.C., Minasian, L., Parnes, H.L., Hsing, A.W., Reichardt, J.K., Hoque, A., Tangen, C.M., Kristal, A.R., Thompson, I.M., Figg, W.D., 2010. Androgen receptor CAG repeat length and association with prostate cancer risk: results from the prostate cancer prevention trial. J Urol 184: 2297-302.

Robbins, C.M., Tembe, W.A., Baker, A., Sinari, S., Moses, T.Y., Beckstrom-Sternberg, S., Beckstrom-Sternberg, J., Barrett, M., Long, J., Chinnaiyan, A., Lowey, J., Suh, E., Pearson, J.V., Craig, D.W., Agus, D.B., Pienta, K.J., Carpten, J.D., 2011. Copy number and targeted mutational analysis reveals novel somatic events in metastatic prostate tumors. Genome Res 21: 47-55.

Robins, D.M., 2004. Multiple mechanisms of male-specific gene expression: lessons from the mouse sex-limited protein (Slp) gene. Prog Nucleic Acid Res Mol Biol 78: 1-36.

Robins, D.M. (2009). The Role of the Androgen Receptor Polyglutamine Tract in Prostate Cancer: In Mice and Men. Androgen Action in Prostate Cancer. D. Tindall and J. Mohler. New York, Springer**:** 269-295.

Robins, D.M., Albertelli, M.A., O'Mahony, O.A., 2008. Androgen receptor variants and prostate cancer in humanized AR mice. J Steroid Biochem Mol Biol 108: 230-6.

Sadar, M.D., 2011. Small Molecule Inhibitors Targeting the "Achilles' Heel" of Androgen Receptor Activity. Cancer Res.

Schauwaers, K., De Gendt, K., Saunders, P.T., Atanassova, N., Haelens, A., Callewaert, L., Moehren, U., Swinnen, J.V., Verhoeven, G., Verrijdt, G., Claessens, F., 2007. Loss of androgen receptor binding to selective androgen response elements causes a reproductive phenotype in a knockin mouse model. Proc Natl Acad Sci U S A 104: 4961-6.

Scher, H.I., Sawyers, C.L., 2005. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. J Clin Oncol 23: 8253-61.

Schoenberg, M.P., Hakimi, J.M., Wang, S., Bova, G.S., Epstein, J.I., Fischbeck, K.H., Isaacs, W.B., Walsh, P.C., Barrack, E.R., 1994. Microsatellite mutation (CAG24-->18) in the androgen receptor gene in human prostate cancer. Biochem Biophys Res Commun 198: 74-80.

Shen, H.C., Coetzee, G.A., 2005. The androgen receptor: unlocking the secrets of its unique transactivation domain. Vitam Horm 71: 301-19.

Stanford, J.L., Just, J.J., Gibbs, M., Wicklund, K.G., Neal, C.L., Blumenstein, B.A., Ostrander, E.A., 1997. Polymorphic repeats in the androgen receptor gene: molecular markers of prostate cancer risk. Cancer Res 57: 1194-8.

Steinkamp, M.P., O'Mahony, O.A., Brogley, M., Rehman, H., Lapensee, E.W., Dhanasekaran, S., Hofer, M.D., Kuefer, R., Chinnaiyan, A., Rubin, M.A., Pienta, K.J., Robins, D.M., 2009. Treatment-dependent androgen receptor mutations in prostate cancer exploit multiple mechanisms to evade therapy. Cancer Res 69: 4434-42.

Taplin, M.E., Bubley, G.J., Ko, Y.J., Small, E.J., Upton, M., Rajeshkumar, B., Balk, S.P., 1999. Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. Cancer Res 59: 2511-5.

Taplin, M.E., Bubley, G.J., Shuster, T.D., Frantz, M.E., Spooner, A.E., Ogata, G.K., Keer, H.N., Balk, S.P., 1995. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. N Engl J Med 332: 1393-8.

Taylor, B.S., Schultz, N., Hieronymus, H., Gopalan, A., Xiao, Y., Carver, B.S., Arora, V.K., Kaushik, P., Cerami, E., Reva, B., Antipin, Y., Mitsiades, N., Landers, T., Dolgalev, I., Major, J.E., Wilson, M., Socci, N.D., Lash, A.E., Heguy, A., Eastham, J.A., Scher, H.I., Reuter, V.E., Scardino, P.T., Sander, C., Sawyers, C.L., Gerald, W.L., 2010. Integrative genomic profiling of human prostate cancer. Cancer Cell 18: 11-22.

Thomas, R.K., Baker, A.C., Debiasi, R.M., Winckler, W., Laframboise, T., Lin, W.M., Wang, M., Feng, W., Zander, T., MacConaill, L., Lee, J.C., Nicoletti, R., Hatton, C., Goyette, M., Girard, L., Majmudar, K., Ziaugra, L., Wong, K.K., Gabriel, S., Beroukhim, R., Peyton, M., Barretina, J., Dutt, A., Emery, C., Greulich, H., Shah, K., Sasaki, H., Gazdar, A., Minna, J., Armstrong, S.A., Mellinghoff, I.K., Hodi, F.S., Dranoff, G., Mischel, P.S., Cloughesy, T.F., Nelson, S.F., Liau, L.M., Mertz, K., Rubin, M.A., Moch, H., Loda, M., Catalona, W., Fletcher, J., Signoretti, S., Kaye, F., Anderson, K.C., Demetri, G.D., Dummer, R., Wagner, S., Herlyn, M., Sellers, W.R., Meyerson, M., Garraway, L.A., 2007. High-throughput oncogene mutation profiling in human cancer. Nat Genet 39: 347-51.

Tomlins, S.A., Laxman, B., Dhanasekaran, S.M., Helgeson, B.E., Cao, X., Morris, D.S., Menon, A., Jing, X., Cao, Q., Han, B., Yu, J., Wang, L., Montie, J.E., Rubin, M.A., Pienta, K.J., Roulston, D., Shah, R.B., Varambally, S., Mehra, R., Chinnaiyan, A.M., 2007. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 448: 595-9.

Wang, Q., Udayakumar, T.S., Vasaitis, T.S., Brodie, A.M., Fondell, J.D., 2004. Mechanistic relationship between androgen receptor polyglutamine tract truncation and androgen-dependent transcriptional hyperactivity in prostate cancer cells. J Biol Chem 279: 17319-28.

Watson, P.A., Chen, Y.F., Balbas, M.D., Wongvipat, J., Socci, N.D., Viale, A., Kim, K., Sawyers, C.L., 2010. Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. Proc Natl Acad Sci U S A 107: 16759-65.

Yu, Z., Wang, A.M., Robins, D.M., Lieberman, A.P., 2009. Altered RNA splicing contributes to skeletal muscle pathology in Kennedy disease knock-in mice. Dis Model Mech 2: 500-7.

Zitzmann, M., Depenbusch, M., Gromoll, J., Nieschlag, E., 2004. X-chromosome inactivation patterns and androgen receptor functionality influence phenotype and social characteristics as well as pharmacogenetics of testosterone therapy in Klinefelter patients. J Clin Endocrinol Metab 89: 6208-17.

Figure Legends

Fig. 1. Structural and functional domains of the AR. Diagrammed are the largely unstructured amino terminus (NTD), zinc-chelating fingers of the DNA binding domain (DBD), and the ligand binding domain (LBD) that is comprised of 12 alpha helices, with dihydrotestosterone (DHT) depicted in the pocket. Relative positions are indicated for the polymorphic Q tract, motifs involved in N-C interaction (FxxLF, WxxLF), and activation functions that depend on ligand binding (AF-2) or are ligand-independent (AF-1, AF-5).

Fig. 2. Mouse prostate cancer parameters vary with AR Q tract length. At top is modeled the inverse correlation between Q tract length and AR transcriptional activity based on transfection assays. In 12 week old TRAMP mice, the percent of prostate dorsal lobe epithelium involved in PIN is greatest in 12Q and least in 48Q mice. Despite this apparently earlier initiation, tumors in 12Q mice progress slowly (leading to a longer time with disease) and have a well-differentiated phenotype; tumors in 48Q mice progress slowly after a long lag to initiation. Following androgen ablation, 12Q mice develop palpable tumors much later than intact littermates, whereas there is no difference in time to palpation for 48Q mice. This qualitative depiction summarizes data in Albertelli et al., 2008.

Fig. 3. Recurring AR mutations from human prostate cancer metastases. Missense mutations found in more than one tumor or multiple times in a single tumor are shown; those occurring multiple times in a single patient are underlined. Color code for treatment groups is as follows: green – flutamide, purple – bicalutamide, orange – both antiandrogens, blue – no endocrine treatment, black – both treated and untreated.

Figure

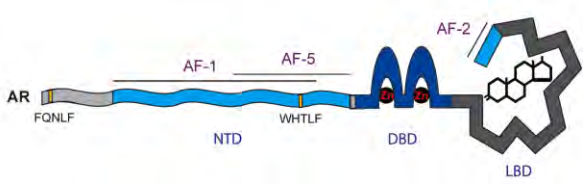

Fig. 1, Robins

Figure 2

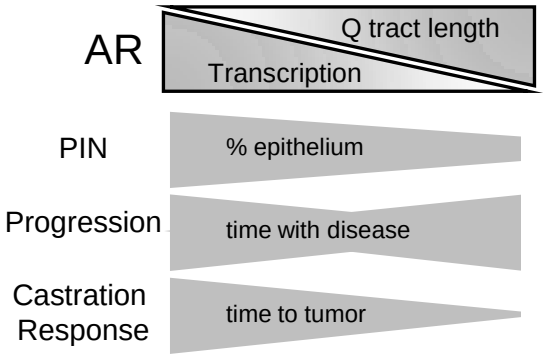

Fig. 2, Robins

Figure 3

Click here to download high resolution image

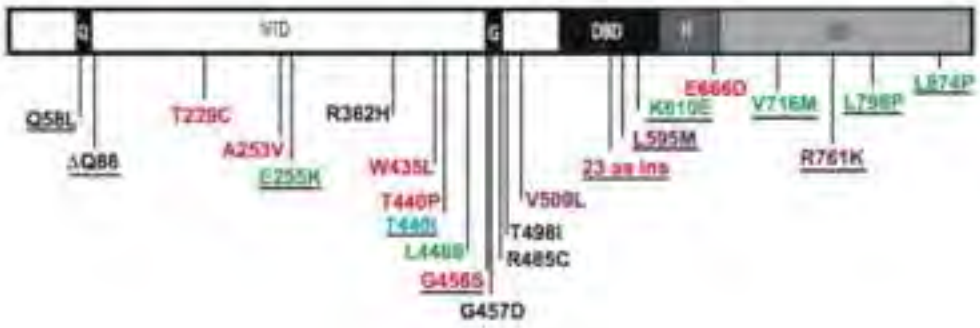

Fig. 3, Robins